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Medical Research Council of Canada

Santé et Bien-être social Canada et
Conseil de recherches médicales du Canada

Laboratory Biosafety Guidelines

Canada

Laboratory Biosafety Guidelines

Medical Research Council of Canada
and
Laboratory Centre for Disease Control
Health Protection Branch
Health and Welfare Canada

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CHAPTER

1

INTRODUCTION

1.1 HISTORY OF MRC GUIDELINES

In February 1977, the Medical Research Council of Canada (MRC) published *Guidelines for the Handling of Recombinant DNA Molecules and Animal Viruses and Cells.* The MRC Guidelines went further than similar documents in the United States of America or the United Kingdom, by addressing the laboratory safety of animal viruses and cells in culture as well as the potential safety problems raised by the application of the new genetic techniques.

MRC undertook this task because of its support of research in universities and their affiliated teaching hospitals. These Guidelines rapidly caused many research institutions to establish biohazard or biosafety committees. The Natural Sciences and Engineering Research Council (NSERC) and the National Research Council of Canada (NRC) adopted and implemented the Guidelines, as did a number of provincial and private research funding agencies. Moreover, the Minister of National Health and Welfare stated that the Guidelines would apply to all research carried out or supported by the federal government. Also, while there was no formal legislative or regulatory enforcement, many industries adopted the Guidelines.

MRC published two revisions in 1979 and 1980, under the advice of its Biohazards Committee. These changes markedly reduced the containment requirements for research using recombinant DNA technology, in line with the rapidly changing perceptions of risk posed by these techniques. Limited changes were made to the containment requirements for the use of animal viruses and cells.

In 1980, MRC published a *Design, Operation and Maintenance Supplement to the Guidelines (the Biohazard Laboratory).*

These documents have been the major national Canadian statements on laboratory safety. However, they have not met all the demands made on them. The growing industrial interest in biotechnology introduced issues such as large-scale fermentations and agricultural and environmental concerns which were not addressed in the MRC Guidelines. Also, even for laboratory research, the

Guidelines did not cover all human pathogens, made only selected reference to animal pathogens, and were silent on plant pathogens. There were also difficulties in reconciling the MRC Guidelines with the needs of clinical diagnostic laboratories. In addition, the use of six levels of containment caused difficulties of coordination with other countries, which had generally described four levels.

For these reasons, and also because Guidelines should be periodically reviewed, MRC undertook to revise them. Because the Laboratory Centre for Disease Control (LCDC) of Health and Welfare Canada (HWC) had developed considerable expertise in biosafety, its collaboration was sought and willingly given. MRC also advised the Interdepartmental Advisory Committee on Biotechnology and its Sub-committee on Biosafety of its intention to revise the Guidelines considering predominantly the handling of pathogens in the laboratory. It was agreed that other governmental agencies would undertake to prepare guidelines or regulations, as deemed appropriate, to meet governmental responsibilities in agricultural, industrial and environmental uses of biotechnology.

MRC and LCDC formed a joint Working Group consisting of the following persons:

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This Working Group notified approximately 118 individuals and organizations of the work it was undertaking, and sought written input; some 49 letters were received. Four meetings led to a draft report which was sent to approximately 15 referees. After revision on the basis of their comments, the report was submitted to MRC and HWC for their approval as a draft to be published for public comment. The public responses were analysed by an editorial sub-committee comprised of Dr. L. Babiuk, Dr. C.M. Johnson-Lussenburg, Mrs. M.E. Kennedy, Dr. J. Penner, Dr. K. Rozee and Dr. P. Stockdale. The needed changes were made and the report was submitted to MRC and HWC for final approval.

1.2 SCOPE OF THE DOCUMENT

The Working Group's objective was to provide a technical document for those who design, build, operate or work in research laboratories in which human pathogens are grown for research or development purposes. The focus of this document is therefore on the use of bacteria, viruses, parasites, fungi and other infectious agents which are

pathogenic to man, in research laboratories that might be found in universities, hospitals and their affiliated institutions, as well as in governmental, industrial, research, diagnostic and teaching laboratories.

If a microbial pathogen is isolated or suspected to be present in a specimen, it then must be handled at the appropriate containment level. Every pathogen isolated is to be handled according to the risk category. As with any choice, this focus leaves areas out of consideration. The Working Group does not cover issues such as pathogens of primary interest to agriculture unless they are also human pathogens or are used in medical research laboratories. However, Agriculture Canada has legislative authority which applies directly to agents in these Guidelines; this is described in this document. The Working Group also does not comment on industrial scale uses of pathogens, nor on deliberate environmental release. These and other areas are left to other agencies in accord with the general understanding with the Interdepartmental Advisory Committee on Biotechnology.

The Working Group expects that the Medical Research Council, Health and Welfare Canada and others will develop the regulatory procedures they feel necessary for implementation of the technical considerations set out in this document.

CHAPTER

2

CONTAINMENT OF BIOHAZARDS

2.1 INTRODUCTION

Bacteria, viruses, fungi or other infectious agents are studied because they cause disease, they can help us understand the natural world, and for many other reasons including the possibility of industrial applications. Since many of the agents can be pathogenic to man, animals or other forms of life, their use poses risks which vary with each agent and the way it is used.

Safety standards are designed to reduce to an acceptable level the risks inherent in the use of dangerous materials. Stringent standards are set for hazardous agents and mild ones for agents which cause only minor problems. Safety standards are therefore compromises designed to allow needed work to proceed without exposing those involved or others to more than minimal risk.

In drafting this document, the Working Group has tried to reach compromises consistent with those already adopted in other countries. Such compromises will always be open to discussion. Some will regard the standards as too stringent, others will take the opposite view.

2.2 SAFETY PRACTICES

The attitudes and actions of those who work in the laboratory determine their own safety, and that of their colleagues and of the community. Laboratory equipment and design can contribute to safety only if they are used properly by people who are genuinely concerned and knowledgeable about safety issues.

The Canada Labour Code requires that each employer provide safe working conditions and that employees be informed about all hazards they will face in the course of their duties. The employee is also given the right to withdraw from the workplace if faced with an unsafe condition. Other federal legislation (Workplace Hazardous Materials Information System – WHMIS) requires that all hazardous substances, including microorganisms, be labelled in a specified manner and that there be a Material Safety Data Sheet (MSDS) available to accompany each hazardous substance. Employers are also

required to provide all training necessary to work with the hazardous substance and to keep a written record of their employee education program. For more information, contact Labour Canada (tel: (613) 997-3520).

The following requirements are basic for any laboratory using infectious or toxic agents.

1. All laboratory personnel and others whose work requires them to enter the laboratory must understand the chemical and biological hazards with which they will come in contact through their normal work in the laboratory, and be trained in appropriate safety precautions and procedures. A laboratory safety manual should be prepared or adopted by the laboratory director that identifies known and potential hazards and specifies practices and procedures to eliminate or minimize such risks. The manual should also contain an emergency response plan. Personnel must be required to know, understand, and follow standard practices and procedures. Training in laboratory safety should be provided by the laboratory director and competence in safe technique must be demonstrated before work is allowed with hazardous agents or toxins.
2. The laboratory must be kept neat, orderly and clean, and storage of materials not pertinent to the work should be minimized.
3. Protective laboratory clothing (uniforms, coats, gowns) must be available, and worn properly fastened by all personnel, including visitors, trainees, and others entering or working in the laboratory. Protective laboratory clothing must not be worn in non-laboratory areas. Suitable footwear with closed toes and heels and preferably with non-slip soles should be worn in all laboratory areas.
4. Safety face and eyewear (e.g. glasses, goggles, face shields, or other protective devices) should be worn when necessary to protect the face and eyes from splashes, impacting objects, harmful substances, UV light, or other rays.
5. Eating, drinking, smoking, storing food or utensils, applying cosmetics, and inserting or removing contact lenses are not permitted in any laboratory work area. Contact lenses should be worn only when other forms of corrective eyewear are not suitable.
6. Oral pipetting is prohibited in any laboratory.

7. Long hair must be tied back or restrained.

8. Hands must be washed before leaving the laboratory and at any time after handling materials known or suspected to be contaminated, even when gloves have been worn.

9. Work surfaces must be cleaned and decontaminated with a suitable disinfectant at the end of the day and after any spill of potentially dangerous material. Loose or cracked work surfaces should be replaced.

10. All technical procedures should be performed in a manner that minimizes the creation of aerosols.

11. All contaminated or infectious liquid or solid materials must be decontaminated before disposal or reuse. Contaminated materials that are to be autoclaved or incinerated at a site away from the laboratory should have the outside disinfected chemically or be double-bagged and then transported to the autoclave or incinerator in durable leakproof containers which are closed and wiped on the outside with disinfectant before being removed from the laboratory.

12. Access to the laboratory should be severely limited or restricted at Levels 3 and 4. Decisions on entry into Level 1 and 2 laboratories should be at the discretion of the laboratory director (e.g. only persons who have been advised of the potential hazards and meet any specific entry requirements such as immunization should be allowed to enter the laboratory area). Special attention should be paid to the possibility that pregnant women or immunocompromised people might work in or enter the laboratory, or that children might enter.

13. Hazard warning signs, indicating the risk level of the agents being used, must be posted outside each laboratory. Where infectious agent(s) used in the laboratory require special provisions for entry, the relevant information must be included in the sign. The agent must be identified, and the name of the laboratory supervisor and other responsible person(s) as well as any special conditions for staff entry must be listed.

14. Gloves should be worn for all procedures that might involve direct skin contact with toxins, blood, infectious materials, or infected animals. Gloves should be removed carefully and decontaminated with other laboratory wastes

before disposal. Reusable gloves must be appropriately decontaminated.

15. Hypodermic needles and syringes should be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Extreme caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles should not be bent or sheared by hand. They should not ordinarily be replaced in the sheath or guard. They should be promptly placed in a puncture-resistant container and decontaminated, preferably by incineration or autoclaving, before disposal.

16. All spills, accidents, and overt or potential exposures must be reported in writing to the laboratory supervisor or acting alternate as soon as circumstances permit; this person should file this report with the appropriate biosafety committee. Appropriate medical evaluation, surveillance, and treatment should be provided as required.

17. When appropriate, baseline serum or other samples for laboratory and other at-risk personnel should be collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.

18. Laboratory workers should be protected against relevant infection by immunization where possible and show immunity.

2.3 USE OF LABORATORY ANIMALS

Naturally occurring or experimentally induced infections in laboratory animals may be transmitted to other laboratory animals, invertebrates and laboratory workers. Animals infected or challenged experimentally with organisms in any of the risk groups may be small (e.g. mice) or large (e.g. livestock), have unique housing requirements (e.g. fish) or have uncharacterized susceptibilities (e.g. gnotobiotes, transgenics). The requirements for maintenance of the animals may differ therefore in scale and degree but the basic principles for microbiological safety will be similar to those outlined in Section 2.2.

In addition to those practices, the following requirements and conditions must be satisfied:

- All aspects involved in the proposed use of animals in research must meet the standards and regulations for the care and maintenance of experimental animals as described by the Canadian Council on Animal Care, relevant provincial legislation and local animal care authorities.
- It should be clear that appropriate species have been selected for the animal experiments.
- The investigator and/or person(s) responsible for the animal experiment must ensure that all those having contact with the animals and waste materials are familiar with and aware of any special precautions and procedures that may be required. Where possible, personnel should be protected by immunization with appropriate vaccines.
- It is essential that all accidents, including animal bites and scratches or cuts from cages or other equipment, be reported and recorded.
- Small laboratory rodents or other small animals that escape from their cages should be killed when captured, their carcasses incinerated and the area should be fully decontaminated. In the event that animals escape the containment perimeter, the relevant authorities must be notified promptly and appropriate action initiated.
- Unexpected illness or deaths among animals must be reported without delay; instructions for dealing with such animals should be available. However, animals should not be touched until instructions are given by the person in charge.

CHAPTER

3

CLASSIFICATION OF BIOLOGICAL AGENTS ACCORDING TO RISK

3.1 GENERAL

Judgements of the inherent risks of a pathogen are made on the basis of such factors as the severity of the disease it causes, the routes of infection, and its virulence and infectivity. This judgement should take into account the existence of effective therapies, possibilities for immunization, the presence of vectors, quantity of agent and whether the agent is indigenous to Canada, possible effects on other species, including plants, or possible economic environmental effects.

With these factors as the prime consideration, organisms are classified according to risk groups, which are analogous to the levels of containment described in Chapter 4. These classifications presume ordinary circumstances in the research laboratory, or growth in small volumes for diagnostic and experimental purposes.

The classifications of biological agents primarily reflect the judgements made on their inherent risks. Section 3.7 lists the general criteria followed. The agents listed in Section 3.8 were chosen because they seem to be frequently used in Canada. Agents not listed should be classified on the basis of similarity to those listed; risk groups for others will be identified on request to MRC or LCDC.

In addition to the severity of disease or other risks caused by a pathogen, the conditions under which an infectious agent is used also concern laboratory safety. Large volumes and high concentrations of agent in growth media evidently pose greater risks than smears of the same agent on a microscope slide. Other unusual manipulations may also increase the hazard. Where deemed appropriate, Section 3.8 includes annotations indicating changes in classification when certain agents are used in defined ways.

3.2 RECOMBINANT DNA AND GENETIC MANIPULATION

Genetic methods such as natural selection, cross breeding, conjugation and transformation have been used for many years to change biological species and organisms. These methods have recently been supplemented by newer and much more powerful ones, of which the best known are the techniques of recombinant DNA. This technology allows scientists to transfer genes between unrelated organisms and species, and has spawned the recent surge in biotechnology.

The initial fear of possible risks arising from organisms altered by this technology led Canada, the United States and Great Britain, among other countries, to develop stringent biosafety guidelines. Experience rapidly showed that the initial fears were not justified. By 1980, many of the containment requirements of 1975-1977 had been removed.

Guidance in how to assess potential risks in recombinant DNA research can only be very general; each case needs individual assessment by the Biohazards Committee at the research institution. It is not realistic to try to define in advance all of the possible genetically engineered organisms which might be created or used in the laboratory. The vast majority of this research involves only the remotest possibility of creating a hazard because the source of the DNA being transferred, the vector and the host are all innocuous. However, some genetic manipulation does raise significant possibility of risk. In general, if none of the components of the genetic manipulation presents any known hazard, and none can be reasonably foreseen in their combination, then no biohazard restrictions are needed. If one of the components of the reaction is hazardous, then, in general, discussion of the containment level required should start at the level appropriate to the known hazard, from which it might be increased or decreased according to such considerations as the particular gene being transferred, the expression of the gene in the recombinant organism, the biological containment offered by the host vector system, envisaged interactions between the gene being transferred and the host vector system and other such factors. In any research with genes coding for hazardous products, host vector systems of limited ability to survive outside the laboratory (i.e. offering biological containment) should be used; their use will reduce the level of containment required.

Genetic manipulation, by deletion of certain functions, can also reduce the risks of pathogenicity of biological

agents. Evidently, deletion mutants that cause them not to reproduce or cause disease in the host will reduce the risk level.

3.3 USE OF MAMMALIAN CELLS IN CULTURE

The biological hazards of mammalian cells arise from the possibility that they might contain or transmit viruses. Cells known or suspected to contain infectious agents, or primary cultures from animals and humans known or reasonably suspected to be infected, should be in the risk group for the suspected agent.

3.4 RISK LEVELS ASSOCIATED WITH THE USE OF LABORATORY ANIMALS

The use of experimental animals and insects poses special problems. Animals can harbour infectious organisms which are acquired naturally. These infections can give rise to a chronic carrier state, or the agent might persist in a latent non-infective form which can be reactivated periodically or as a result of certain stimuli. If the possibility that such an agent may be excreted by an animal during the course of an experiment cannot be excluded, all those animals should be kept at a containment level appropriate to the risk.

Animals may also be deliberately inoculated with organisms in each of the four risk groups or with viable materials (i.e. transformed cells) suspected of containing these organisms. Under these circumstances, the animals should be kept at the containment level appropriate to the risk of the organism, recognizing that, in some cases, in vivo work may increase that risk.

In all situations, it is the responsibility of the scientist and the local biosafety/biohazard committee, in consultation with the Animal Care authorities, to determine the risk levels inherent in the proposed research.

3.5 THE ANIMAL DISEASES AND PROTECTION ACT

The Animal Diseases and Protection Act and Regulations, 1987, give Agriculture Canada the legislative authority to control the distribution and use of any pathogen which may cause infectious or contagious disease in animals. This control would also apply to materials of animal origin which contain potential pathogens. In practical terms, this means that Agriculture Canada's approval must be obtained for the importation of every animal pathogen. It may also establish, for any animal pathogen, the conditions under which work will be carried out and restrict distribution of the organism to a certain specified person or laboratory. These pathogens are noted and listed in Table 1 and Section 3.8. If an agent was originally brought into Canada under an import permit which restricts its distribution, further approval must be obtained before transferring it to another scientist or laboratory. Any recombinant organism could fall into a restrictive category and it may be necessary to prove to the appropriate authorities that its use or release into the environment would not constitute a risk to any animal. Thus, for any organism, it will be necessary to consider not only the risk to human health, but also the level of containment needed to prevent its escape into the environment where it might infect some indigenous animal species.

3.5.1 NON-INDIGENOUS AGENTS

A partial list of animal disease agents considered as not indigenous to Canada is provided in Table 1. The importation of all micro-organisms pathogenic to animals is subject to control under the Animal Diseases and Protection Act and its Regulations. A permit for the importation and laboratory use of the organisms listed must be obtained from the Chief, Veterinary Biologics and Biotechnology, Health of Animals Directorate, Agriculture Canada, 3851 Fallowfield Road, Box 11300, Station H, Nepean, Ontario K2H 8P9 (tel: (613) 998-9320).

Table

1

Agents not indigenous to Canada

Agents not indigenous including the following require an importation permit from Agriculture Canada. In addition, Agriculture Canada will establish conditions under which these organisms are used and maintained.

Bacteria

Mycoplasma agalactiae
Mycoplasma mycoides
Rickettsia ruminantium

Parasites

Besnoitia besnoiti
Theileria annulata
Theileria bovis
Theileria hirci
Theileria lawrencei
Theileria parva
Trypanosoma equiperdum
Trypanosoma evansi
Trypanosoma vivax

Viruses

Bornaviridae
- Borna disease virus

Bunyaviridae
- Nairobi sheep disease virus
- Rift Valley fever virus

Caliciviridae
- Swine vesicular disease
- Vesicular exanthema virus

Herpesviridae
- Pseudorabies virus

Iridoviridae
- African swine fever virus

Orthomyxoviridae
- Fowl plague virus

Paramyxoviridae
- Rinderpest, Newcastle disease virus (mesogenic, velogenic strains),
- Peste des petits ruminants

Picornaviridae
- Genus Aphthovirus: Foot-and-mouth disease virus
- Genus Enterovirus: Teschen disease virus

Poxviridae
- Chordopoxvirinae (poxviruses of vertebrates), Smallpox (Alastrim)
- Genus Capripoxvirus
  - Sheeppox
  - Goatpox
  - Lumpy skin disease
- Genus Suipoxvirus
  - Swinepox
  - Camelpox virus

Reoviridae
- Genus Orbivirus
  - Bluetongue virus
  - African horsesickness virus

Rhabdoviridae
- Genus Vesiculovirus
  - Ephemeral fever virus
  - Vesicular stomatitis virus (Animal inoculation)

Togaviridae
- Hog Cholera virus
- Louping Ill virus (Animal inoculation)
- Wesselsbron disease virus

3.6 IMPORTATION AND TRANSPORTATION OF INFECTIOUS SUBSTANCES

3.6.1 IMPORTATION

Permits are required for the importation of all infectious substances into Canada regardless of whether they infect humans, animals or plants. The importation of infectious agents which are predominantly pathogenic to animals is

regulated by means of the Animal Diseases and Protection Act and Regulations administered by Agriculture Canada. It is also necessary to obtain permission to transfer listed pathogens within Canada from one scientist or laboratory to another. Many pathogens were originally brought into Canada under a Canadian import permit which restricts their distribution and which may stipulate special conditions for their use. Other pathogens have been brought into Canada without the required import permit or may have been placed into a restrictive usage category subsequent to their original entry. Certain agents listed in Section 3.8 of this document require permits jointly issued by Agriculture Canada and Health and Welfare Canada. They are individually noted in the table. Requests for single-entry and long-standing permits to import infectious substances affecting humans should be directed to Chief, Office of Biosafety, Laboratory Centre for Disease Control, Ottawa, Ontario K1A 0L2 (tel: (613) 957-1779). Information about the status of veterinary pathogens and permits may be obtained by applying in writing to the Chief, Veterinary Biologics and Biotechnology, Health of Animals Directorate, Agriculture Canada, 3851 Fallowfield Road, Box 11300, Station H, Nepean, Ontario K2H 8P9 (tel: (613) 998-9320).

3.6.2 TRANSPORTATION

The careful handling, transfer and shipment of diagnostic specimens and infectious agents is absolutely essential if Canada is to maintain an effective health care system. Transportation methods must minimize risks to employees of the carrier, the public and the staff of the receiving laboratory. Hazards are compounded by improper packaging; a broken specimen container may lead to contamination of both laboratory and non-laboratory personnel, and an improperly labelled package may be opened inadvertently by secretarial, clerical or other uninformed staff.

In Canada, effective July 1, 1985, Transport Canada has become responsible for regulations concerning the transportation of dangerous goods. Any person handling, offering for transport or transporting dangerous goods must comply with the Transportation of Dangerous Goods Act and Regulations, Registration SOR 85-77. More specifically, Sections 7.15-7.19 of the Regulations require that, for any quantity of infectious substance, a summary emergency response plan be filed with the Director General of the Transport Dangerous Goods Directorate of Transport Canada, Canada Building, 344 Slater Street, 13th Floor, Ottawa, Ontario K1A 0N5.

In these Regulations, three groups of infectious substances are defined with set standards for packaging, labelling, and documentation. Penalties for failing to comply with the Regulations are severe. Every institution involved in the transportation of dangerous goods should obtain a copy of the Regulations† and make them readily available to individuals planning to transport infectious substances. The Transportation of Dangerous Goods Act and Regulations may be purchased from the Canadian Government Publishing Centre, Department of Supply and Services, Ottawa, Ontario K1A 0S9, or at any bookstore which sells government publications. Further information concerning the Transportation of Dangerous Goods Act and Regulations may be obtained by contacting the Chief of Response and Planning at (613) 991-9396.

In addition, information may be routinely obtained from the Canadian Transport Emergency Centre (CANUTEC) during working hours at (613) 992-4624 and IN AN EMERGENCY round the clock at (613) 996-6666.

The efficient transfer of infectious substances requires good coordination between the sender, carrier, and receiver to ensure safe and prompt transport and arrival in proper condition. It is important that the sender make advance arrangements with the carrier to ensure that specimens will be accepted and promptly processed. In addition, the sender must prepare the appropriate dispatch documents according to the Transportation of Dangerous Goods Act and Regulations. The sender should also forward all transportation data to the receiver. No infectious substances should be dispatched before advance arrangements have been made between the sender, the carrier and the receiver, or before the receiver has confirmed with national authorities that the substance can be imported legally (see Importation, Section 3.6.1) and that no delay will be incurred in the delivery of the consignment to its destination.

3.7 CRITERIA FOR CLASSIFICATION OF INFECTIVE PATHOGENS BY RISK GROUP

Risk Group 1 (low individual and community risk)
A microorganism that is unlikely to cause disease in healthy workers or animals.

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† Amendments to the regulations include SOR 85-314, SOR 85-585, SOR 85-609, SOR 86-526, SOR 87-186, SOR 87-335, SOR 89-39, and SOR 89-294.

Risk Group 2 (moderate individual risk, limited community risk)
A pathogen that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available and the risk of spread is limited.

Risk Group 3 (high individual risk, low community risk)
A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that can be treated by antimicrobial or antiparasitic agents.

Risk Group 4 (high individual risk, high community risk)
A pathogen that usually produces very serious human or animal disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa directly or indirectly, or by casual contact.

3.8 CATEGORIES OF PATHOGENS

As a general precaution, the risk group for agents should be raised when manipulation may result in the production of infectious droplets and aerosols. Agents of similar pathogenic characteristics not included in these lists should be considered in the same risk category. It must also be understood that this is not a complete list – many agents are referred to in the literature by a variety of names and before assuming that an unlisted organism is classified in Risk Group 1, the laboratory worker must fully verify its characteristics.

*** Agents noted with a single asterisk require a joint importation permit from Agriculture Canada and Health and Welfare Canada. Agriculture Canada may also require additional conditions for the use or importation of these agents (see Sections 3.5 and 3.6).**

3.8.1 RISK GROUP 1 AGENTS: REQUIRING CONTAINMENT LEVEL 1

Risk Group 1 (low individual and community risk)
This group includes those microorganisms, bacteria, fungi, viruses and parasites, which are unlikely to cause disease in healthy workers or animals.

3.8.2 RISK GROUP 2 AGENTS: REQUIRING CONTAINMENT LEVEL 2

Risk Group 2 (moderate individual risk, limited community risk)
A pathogen that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to healthy laboratory workers, the community, livestock, or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available and the risk of spread is limited.

Risk Group 2 Bacteria

Actinobacillus * – all species

Bacillus cereus

Bartonella bacilliformis

Bordetella pertussis, B. parapertussis and *B. bronchiseptica*

Borrelia recurrentis, B. vincentii and *B. burgdorferi*

Campylobacter spp. *(C. fetus*, C. jejuni*)*

Clostridium botulinum, Cl. chauvoei*, Cl. difficile, Cl. haemolyticum*, Cl. histolyticum*, Cl. novyi*, Cl. septicum*, Cl. sordellii*, Cl. tetani**

Corynebacterium diphtheriae, C. haemolyticum, C. pseudotuberculosis*, C. pyogenes**

Edwardsiella tarda

*Erysipelothrix insidiosa**

Escherichia coli enterotoxigenic/invasive/hemorrhagic strains*

Francisella tularensis Type B, (biovar palaearctica), *F. novocida*

*Fusobacterium necrophorum**

Haemophilus influenzae, H. ducreyi

Legionella spp.

*Leptospira interrogans** – all serovars

Listeria monocytogenes

Mycobacteria – all species (except *M. tuberculosis, M. bovis* (non-BCG strain), *M. avium* which are at Level 3)

Neisseria gonorrhoeae, N. meningitidis

Nocardia asteroides, N. brasiliensis

*Pasteurella**, all species (except *P. multocida* type B in Level 3)

Salmonella enterica (S. choleraesuis)

Salmonella enterica serovar arizonae *(Arizona hinshawii)*

Salmonella enterica ser. gallinarum-pullorum* *(S. gallinarum-pullorum)*

Risk Group 2

Salmonella enterica ser. meleagridis* *(S. meleagridis)*

Salmonella enterica ser. paratyphi B *(S. paratyphi B)* (Schottmülleri)

Salmonella enterica ser. typhi *(S. typhi)*

Salmonella enterica ser. typhimurium *(S. typhimurium)*

Shigella boydii, S. dysenteriae, S. flexneri, S. sonnei

*Staphylococcus aureus**

Streptobacillus moniliformis

Streptococcus spp. (Lancefield Groups A, B, C, D, G)

Treponema carateum, T. pallidum and *T. pertenue*

Vibrio cholerae (incl. El Tor), *V. parahaemolyticus*

Yersinia enterocolitica, Y. pseudotuberculosis

Risk Group 2 — Chlamydia

*Chlamydia psittaci**, *C. trachomatis*

Risk Group 2 — Mycoplasma

(except those strains pathogenic to animals)*

Mycoplasma pneumoniae, M. hominis

Ureaplasma urealyticum

Risk Group 2 — Fungi

Cryptococcaceae

Candida albicans

Cryptococcus neoformans

Moniliaceae

Aspergillus flavus

Aspergillus fumigatus

Epidermophyton floccosum

Microsporum spp.

Sporothrix schenckii

Trichophyton spp.

Risk Group 2 — Viruses

** Arthropod-borne viruses are identified with a double asterisk. Only those viruses which may be associated with human or animal disease have been included in this list.

Adenoviridae

Adenoviruses, all serotypes

Arenaviridae

Lymphocytic choriomeningitis virus (laboratory-adapted strains)

Tacaribe virus complex: Tamiami, Tacaribe, Pichinde

Risk Group 2

- Bunyaviridae**
  - Genus Bunyavirus
    - Bunyamwera and related viruses
    - California encephalitis group, including LaCrosse, Lumbo and snowshoe hare
  - Genus Phlebovirus
    - All species except Rift Valley fever virus (see Table 1)
- Caliciviridae – all isolates
- Coronaviridae
  - Human coronavirus, all strains
  - Transmissible gastroenteritis virus of swine*
  - Hemagglutinating encephalomyelitis virus of swine*
  - Mouse hepatitis virus
  - Bovine coronavirus*
  - Feline infectious peritonitis virus
  - Avian infectious bronchitis virus*
  - Canine, Rat and Rabbit coronaviruses*
- Flaviviridae**
  - Yellow fever virus (17D vaccine strain)
  - Dengue virus (serotypes 1,2,3,4)
  - Kunjin virus
- Hepadnaviridae
  - Hepatitis B virus
- Herpesviridae
  - Alphaherpesvirinae – all isolates (except Herpes B virus, which is in Level 4 and Pseudorabies (see Table 1))
    - Genus Poikilovirus: all isolates
    - Genus Varicellavirus: all isolates
    - Other herpesviruses unassigned as yet: includes infectious bovine rhinotracheitis, equine coital exanthema virus, feline infectious rhinotracheitis, chicken infectious laryngotracheitis virus, channel catfish virus, etc.
  - Betaherpesvirinae
    - Genus Cytomegalovirus: all isolates
    - Genus Muromegalovirus: all isolates
  - Gammaherpesvirinae
    - Genus Lymphocryptovirus: all isolates (except *H. ateles* and *H. saimiri* in Level 3)
    - Genus Thetalymphocryptovirus: all isolates

Risk Group 2

Orthomyxoviridae

- Genus Influenzavirus: Influenza virus type A, all isolates
  Influenza virus type B, all isolates
  Influenza virus type C, all isolates

Papovaviridae

- Genus Papillomavirus: all isolates
- Genus Polyomavirus: all isolates

Paramyxoviridae

- Genus Paramyxovirus: all isolates
- Genus Morbillivirus: all isolates (except Rinderpest – see Table 1)
- Genus Pneumovirus: all isolates

Parvoviridae

- Genus Parvovirus: all isolates

Picornaviridae

- Genus Aphthovirus – See Table 1
- Genus Enterovirus – all isolates (see Table 1 for restrictions)
- Genus Cardiovirus – all isolates
- Genus Rhinovirus – all isolates

Poxviridae (see Table 1 for restrictions)

- Chordopoxvirinae (poxviruses of vertebrates)
  - Genus Orthopoxvirinae – all isolates (except Variola and Monkeypox in Level 4)
  - Genus Leporipoxvirus – all isolates
  - Genus Avipoxvirus – all isolates
  - Genus Suipoxvirus: Swinepox (see Table 1 for restrictions)
  - Genus Parapoxvirus – all isolates
  - All other ungrouped poxviruses of vertebrates

Reoviridae

- Genus Orbivirus – all isolates (see Table 1 for restrictions)
- Genus Rotavirus – all isolates

Retroviridae

- Oncovirinae
  - Genus Oncornavirus C
    - Subgenus Oncornavirus C avian – all isolates
    - Subgenus Oncornavirus C mammalian – all isolates including HTLV-I, HTLV-II only as non-cultured specimens
  - Genus Oncornavirus B – all isolates

Risk Group 2

Retroviridae *(cont)*

- Lentivirinae – all isolates including HIV-I, HIV-II only as non-cultured specimens
- Spumavirinae – all isolates

Rhabdoviridae

- Genus Vesiculovirus – all isolates (see Table 1 for restrictions)
- Genus Lyssavirus: Rabies virus (Fixed virus)

Togaviridae

- Genus Alphavirus**
  - Semliki forest virus
  - Sindbis
  - Chikungunya (high-passage strains)
  - O'Nyong-Nyong
  - Ross river virus
  - Venezuelan equine encephalitis (Strain TC-83 only)
- Genus Rubivirus: Rubella virus
- Genus Pestivirus
  - Bovine virus diarrhoea
  - Border disease virus
- Genus Arterivirus
  - Equine arteritis virus

Unclassified viruses

- Norwalk viruses
- Non-A, non-B Hepatitis Viruses
- Delta Hepatitis Virus
- Chronic infectious neuropathic agents (CHINAs):
  - Kuru, Creutzfeldt-Jakob agent (Special precautions and disinfectants)
  - Scrapie

Risk Group 2

Parasites

Infective stages of the following parasites have caused laboratory infections by ingestion, skin or mucosal penetration or accidental injection. Preparations of these parasites known to be free of infective stages do not require this level of containment. Most of these organisms do not need to be worked on in a biological safety cabinet but the other constraints of Level 2 containment should be observed.

Work with agents identified as (+) should be conducted in a biological safety cabinet (BSC) or its equivalent.

Risk Group 2

Protozoa –

Babesia microti
Babesia divergens
Balantidium coli
Cryptosporidium spp.+
Entamoeba histolytica
Giardia spp. (mammalian)
Leishmania spp. (mammalian)+
Naegleria fowleri+
Plasmodium spp. (human or simian)
Pneumocystis carinii
Toxoplasma gondii+
Trypanosoma brucei, T. cruzi+

Helminths

Nematodes –

Ancylostoma duodenale
Angiostrongylus spp.
Ascaris spp.
Brugia spp.
Loa loa
Necator americanus
Onchocerca volvulus
Strongyloides spp.
Toxocara canis
Trichinella spp.*
Trichuris trichiura
Wuchereria bancrofti

Cestodes –

Echinococcus (gravid segments)+
Hymenolepis diminuta
Hymenolepis nana (human origin)
*Taenia saginata**
*Taenia solium**

Trematodes –

Clonorchis sinensis
Fasciola hepatica
Opisthorchis spp.
Paragonimus westermani
Schistosoma haematobium
Schistosoma japonicum
Schistosoma mansoni

3.8.3 RISK GROUP 3 AGENTS: REQUIRING CONTAINMENT LEVEL 3

Risk Group 3 (high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that can be treated by antimicrobial or antiparasitic agents.

Risk Group 3 — Bacteria

*Bacillus anthracis**

Brucella – all species*

*Chlamydia psittaci** – avian strains only

*Coxiella burnetii**

Francisella tularensis, type A (biovar tularensis)

*Mycobacterium avium**; *M. bovis** (non-BCG strains); *M. tuberculosis**

Pasteurella multocida, type B*

*Pseudomonas mallei**; *P. pseudomallei**

Rickettsia – all species

Yersinia pestis

Risk Group 3 — Fungi

Moniliaceae

Blastomyces dermatitidis

Coccidioides immitis

Histoplasma capsulatum including var. *duboisii*

*Paracoccidioides brasiliensis**

Risk Group 3 — Viruses

Viruses are grouped within Family and/or Genus. Arthropod-borne viruses are identified with a double asterisk.

Arenaviridae

Lymphocytic choriomeningitis virus, neurotropic strains

Bunyaviridae

Unclassified Bunyavirus

Hantaan, Korean hemorrhagic fever and epidemic nephrosis viruses

Risk Group 3

- Flaviviridae**
  - Yellow fever virus (Wild type)
  - St. Louis encephalitis virus
  - Japanese encephalitis virus
  - Murray Valley encephalitis virus
  - Powassan
- Herpesviridae
  - Gammaherpesvirinae
    - Genus Rhadinovirus: *Herpesvirus ateles; Herpesvirus saimiri*
- Retroviridae
  - Oncovirinae
    - Genus Oncornavirus C
      - Human T-cell leukemia/lymphoma virus (HTLV-I, HTLV-II, if cultured)
    - Genus Oncornavirus D
      - Mason-Pfizer monkey virus
      - Viruses from primates
  - Lentivirinae
    - Human immunodeficiency viruses (HIV – all isolates if cultured)
- Rhabdoviridae
  - Genus Vesiculovirus (see Table 1 for restrictions)
    - Piry
  - Genus Lyssavirus
    - Rabies virus (Street virus)*
- Togaviridae
  - Genus Alphavirus**
    - Eastern equine encephalitis virus*
    - Chikungunya (recent isolates)
    - Venezuelan equine encephalitis (except Strain TC-83)*
    - Western equine encephalitis*
- Unclassified Viruses
  - Chronic infectious neuropathic agents (CHINAs):
    - Kuru, Creutzfeldt-Jakob agent (also listed under Level 2; level of the suspected agent depends on the nature of the manipulations and the amount of sera, bio/necropsy materials handled).

Risk Group 3

Parasites

None

3.8.4 RISK GROUP 4 AGENTS: REQUIRING CONTAINMENT LEVEL 4

Risk Group 4 (high individual risk, high community risk)
A pathogen that usually produces very serious human or animal disease, often untreatable, and may be readily transmitted from one individual to another, or from animal to human or vice-versa directly or indirectly, or by casual contact.

Risk Group 4 — Bacteria

None

Risk Group 4 — Fungi

None

Risk Group 4 — Viruses

Arenaviridae
- Lassa, Junin, Machupo viruses

Bunyaviridae**
- Genus Nairovirus
  - Crimean-Congo hemorrhagic fever

Filoviridae
- Marburg virus
- Ebola virus

Flaviviridae**
- Tick-borne encephalitis complex, including –
  - Russian Spring-Summer Encephalitis
  - Kyasanur forest virus
  - Omsk hemorrhagic fever virus

Herpesviridae
- Alphaherpesvirinae
  - Genus Simplexvirus: Herpes B virus (Monkey B virus)

Poxviridae
- Genus Orthopoxvirinae
  - Variola
  - Monkeypox

Risk Group 4 — Parasites

None

CHAPTER

4

PHYSICAL CONTAINMENT LEVELS

Four levels of containment (1-4) appropriate to the four risk groups for infectious agents are defined below. These levels of containment are to be regarded as appropriate for most laboratory-scale uses of the particular agents. It remains the responsibility of the principal investigator or laboratory director and the institution to require a higher level of containment for specific manipulations, if these appreciably increase the hazard of infection.

Containment Level 1

4.1 CONTAINMENT LEVEL 1

This level applies to the basic laboratory for the handling of Risk Group 1 agents. Level 1 requires no special design features beyond those suitable for a well designed and functional laboratory. Containment cabinets are not required. Work may be done on an open bench top and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

4.1.1 PHYSICAL REQUIREMENTS

- A room separated from public areas by a door is required. There are no particular restrictions on locating the facility near public or heavily travelled corridors; however, doors should remain closed.
- Coatings on walls, ceilings, furniture, and floors should be cleanable. Windows that can be opened should not be near working areas or containment equipment and should be equipped with fly screens.
- There are no special air handling requirements beyond those concerned with proper functioning of the biological safety cabinets, if used, and those required by building codes.
- Handwashing facilities must be provided, preferably near the point of exit to public areas.

Containment Level 1

- Separate hanging areas should be provided for street clothing and laboratory coats.
- Eye wash stations may be required by local statute.

4.1.2 OPERATIONAL REQUIREMENTS

Basic safety practices as described in Chapter 2 must be followed.

In addition, where chemical disinfection procedures are practised, effective concentrations and contact times must be employed. Chemical disinfectants used to decontaminate materials to be removed from the laboratory must be replaced regularly.

Containment Level 2

4.2 CONTAINMENT LEVEL 2

Containment Level 2 is suitable for work with agents in Risk Group 2. In addition to the requirements of containment Level 1, the following are required:

4.2.1 PHYSICAL REQUIREMENTS

- The laboratory should be located away from public areas, general offices, and patient care areas.
- A biohazard sign with appropriate information must be posted on the entrance to the laboratory.
- Laboratory furnishings should be constructed with special attention to the use of impervious and readily cleanable work surfaces.
- Coat hooks must be provided for laboratory coats near the exit.
- An autoclave must be available in or near the laboratory.
- Laboratory doors should be self-closing.

4.2.2 OPERATIONAL REQUIREMENTS

- Class I or II biological safety cabinets (see Appendix B) are required for all manipulations involving the agent which may create an aerosol. The biological safety cabinet must have been tested and certified within the previous 12 months according to accepted standards (see Appendix B).
- Air from these cabinets may be recirculated to the room only after passage through a high-efficiency particulate air (HEPA) filter.

Containment Level 2

- Centrifugation must be carried out using closed containers or safety heads or sealed cups. These should be opened only in the biological safety cabinet described above.
- Animals or insects which have been experimentally infected must remain in the laboratory or appropriate animal containment facility.
- An emergency plan for handling spills of infectious materials must be developed and be ready for use whenever needed.
- Vacuum lines used for work involving the agent must be protected from contamination by HEPA filters or equivalent equipment.
- A laboratory coat to be worn only in the laboratory area is required. Coats that fasten on the front are permissible. These coats shall not be worn outside the containment laboratory.
- Special care should be taken to avoid contamination of the skin with infectious materials; gloves should be worn when handling infected animals or when skin may be exposed to infectious materials.
- Contaminated glassware must not leave the facility; decontamination must be carried out using procedures demonstrated to be effective. If there is no autoclave or incinerator in the laboratory, contaminated materials must be disinfected chemically or double bagged and transported to the autoclave or incinerator in durable, leakproof containers which are closed and wiped on the outside with disinfectant before leaving the laboratory.
- Service personnel and cleaning staff who enter the facility must be informed of the hazards that might be encountered. Cleaning staff should clean only the floors. The laboratory personnel have the responsibility for rendering the facility safe for routine cleaning. Periodic intensive cleaning must be done at regular intervals. Cleaning and maintenance staff may receive appropriate immunization and medical surveillance if appropriate.

Containment Level 3

4.3 CONTAINMENT LEVEL 3

Containment Level 3 is suitable for work with agents in Risk Group 3. The operational requirements for the Level 3 laboratory are substantially greater than those for Levels 1 and 2 and the laboratory staff must receive specific training in the safe handling and manipulation of the agents used in this laboratory.

Containment Level 3

A Level 3 containment laboratory requires specialized design and construction. Those responsible for biosafety in an institution should maintain close control and seek expert advice for all phases of design, construction, commissioning, operation and maintenance, and annual testing.

4.3.1 PHYSICAL REQUIREMENTS

The following are required in addition to Levels 1 and 2:

- The laboratory must be located away from general work areas and have controlled access from other areas. This is accomplished by entry through a lockable changing room with self-closing doors. A body shower should be provided within the containment perimeter.
- The laboratory must be held at a negative pressure relative to the surrounding areas at all times such that a directional airflow is created by air ingressing through all entry and exit areas. The air discharged from the laboratory cannot be recirculated back into either the air supply system of the laboratory itself or into the building or adjacent buildings. Provided there is a dedicated sealed exhaust system, air may be exhausted from the laboratory to the exterior of the building without HEPA filtration. At the discharge point the exhausted air must be dispersed away from air intakes or populated areas. However, when the air is not exhausted by means of a dedicated exhaust system, it must be passed through a HEPA exhaust housing before discharging into the main building exhaust air ventilation system. This exhaust housing must be designed to allow in situ decontamination and must pass annual testing and certification by aerosol challenge and scan techniques. A control system must be provided to ensure that the Level 3 laboratory does not become positively pressurized relative to the surrounding area. When the supply air is not provided by a dedicated system, back draft dampers or HEPA filters must be installed in the supply system.
- Biological safety cabinets must be installed in a manner which does not interfere with the air balance of the cabinet or room. Thimble unit connections are recommended (see Appendix B).
- The laboratory must have a dedicated handwashing sink with foot, knee or automatic controls, located near the exit.
- The laboratory should have an autoclave located within the work zone. Where this is not possible, contaminated materials may be autoclaved or

incinerated outside the laboratory if they are chemically disinfected first or double bagged then transported to the autoclave or incinerator in a properly identified, closed, unbreakable and leakproof container.

- Laboratory furnishings should be kept to a minimum, be readily cleanable, and resistant to chemical disinfectants.
- All penetrations for services in the floors, walls, and ceiling of the laboratory must be sealed. The air exhaust system should be provided with a manual damper that may be closed as required to permit gas decontamination.
- Water supplied to the laboratory must be provided with back flow preventers.
- Sink and floor drains from this suite should be piped separately to the main building drain and be appropriately labelled. Floor drains are not generally recommended. Infectious materials must never be placed in sinks or floor drains.
- In animal care facilities for small animals, the disposal of wastes will not differ from other contaminated laboratory materials. Large animals producing quantities of infectious wastes require special facilities which must be individually approved.
- Portable vacuum pumps must be fitted with in-line HEPA filters or equivalent equipment.
- Laboratory windows must be sealed and unbreakable.

4.3.2 OPERATIONAL REQUIREMENTS

The following are required in addition to those stated in Levels 1 and 2:

- Laboratory staff must be fully trained in the handling of pathogenic material and in the use of safety equipment, disposal techniques, handling of contaminated waste, and emergency response.
- Staff are required to change into dedicated solid front laboratory clothing on entry to the facility. This clothing must be removed on completion of work and autoclaved prior to laundering.
- Personal effects may not be taken into or stored in the laboratory.
- Gloves must be worn when handling infective or potentially infective materials or animals.
- All activities involving infectious materials are conducted in biological safety cabinets or other

appropriate combinations of personal protective or physical containment devices.

- Centrifugation must be carried out in closed containers using sealed safety heads or centrifuge cups which are loaded and unloaded in the biological safety cabinet.
- Effective disinfectants must be available at all times in the laboratory.
- An effective insect and rodent control program must be in effect.
- Written protocols must be provided and posted within the laboratory outlining waste disposal, disinfection procedures, and emergency response.
- The facility must have a medical surveillance program which includes serum storage for all personnel working in the containment laboratory.
- The laboratory must have a reporting system for accidents and exposures to infective agents or other unusual occurrences in the operation of the laboratory.
- Authorized maintenance personnel must abide by the same operational protocols as laboratory staff and be accompanied by laboratory personnel.
- The Level 3 facility and its systems must be tested for containment capability upon completion of construction and at least annually thereafter.

4.4 CONTAINMENT LEVEL 4

Containment Level 4 is the highest level of containment and represents a geographically isolated unit functionally independent of other areas.

This level of containment requires an air lock for entry and exit, Class III biological safety cabinets, and/or positive pressure ventilated suits, a laboratory support area, and a separate ventilation system in addition to the physical and operational requirements of Levels 1 to 3.

4.4.1 PHYSICAL REQUIREMENTS

- The laboratory must be physically separated from other laboratories or consist of an isolated zone which is monolithic in construction with all penetrations to floors, walls, and ceilings sealed with non-shrinking sealant.
- The laboratory must be designed to accommodate a minimum of two staff at all times.

Containment Level 4

- Entry must be through a double airlock door system which is self-closing with doors which are electrically interlocked.
- Change rooms must be contiguous with the perimeter of the structure and have a personnel shower.
- All drain traps must be kept filled with an effective disinfectant and connected to a liquid waste effluent system.
- All air access to any sewer and ventilation lines must be fitted with HEPA filters.
- All gas services must be fitted with HEPA filters, equivalent equipment, or back-flow preventers to prevent egress of contaminated material.
- The water supply systems for the laboratory must be provided with back-flow preventers.
- All windows must be sealed and provided with break-resistant glass or plastic panes.
- The laboratory must be provided with a double-door autoclave serviceable from outside the facility. The autoclave should have read-out charts inside and outside the containment laboratory.
- Dunk tank(s) which are chemically resistant and of a suitable size for the passage of anticipated laboratory materials are required.
- A dedicated handwashing sink with foot, knee or automatic controls must be located at the exit of each laboratory room in the facility.
- The facility must be equipped with a two-way intercom system.
- All liquid effluents from the facility must enter a waste effluent treatment system for sterilization which is mechanically and biologically monitored.
- The facility must be ventilated by a dedicated, sealed supply and exhaust air system which is not recirculated. Exhaust air must be passed through at least 2 HEPA filters mounted in series in sealed housings designed to allow in situ decontamination and testing by aerosol challenge techniques. Supply and exhaust ventilation systems must be designed to maintain directional (inward) airflows and pressure differentials, and interlocked to prevent pressurization in the event of exhaust fan failure. The supply air system must be equipped with a HEPA filter. The facility must be fully equipped with manometers and audible alarms capable of being monitored by both laboratory and maintenance staff.

Containment Level 4

- The laboratory must be provided with Class I, II or III certified and tested biological safety cabinets which must be installed in a manner that does not interfere with the air balance of the cabinet or room.
- If a positive-pressure-ventilated-suit type of operation is used, a life-support system with full alarming, backup breathing air, emergency power and a chemical shower facility are required. Positive-pressure suits should be used whenever agents are worked with outside a Class III cabinet.
- Alarm systems must be on a separate electrical circuit with emergency backup.
- A laboratory support area outside the Level 4 containment facility is required for all non-hazardous laboratory manipulations.

4.4.2 OPERATIONAL REQUIREMENTS

- Only fully authorized personnel may enter the Level 4 containment laboratory. A logbook signed by all personnel must be maintained.
- At all times when someone is working in the laboratory, there must be a second competent person present in the laboratory who is able to assist in case of emergency.
- Protective clothing, gloves, and impermeable footwear are required. No street clothing will be worn under the protective clothing. On exit from the area, personnel will shower and redress in street clothing.
- Small laboratory animals or insects under experimentation must be held in this area in ventilated cabinets having all output air filtered.
- Large animals require specialized care and handling not dealt with by these guidelines.
- All material shall be removed from the contaminated zone through an autoclave or placed in a double, unbreakable, sealed container, the outside of which will be disinfected. Where equipment and apparatus are not compatible with heat sterilization, materials may be removed via dunk tanks or air locks with suitable decontamination procedures.
- All manipulations with agents in containment Level 4 must be performed in a Class III biological safety cabinet (see Appendix A) or in Class I or II biological safety cabinets used in conjunction with one-piece, positive-pressure-ventilated suits when human pathogens are involved. When foreign animal disease

pathogens are handled in a Level 4 facility, suits need not be used and work can be done in a Class II safety cabinet provided that their pathogenicity for humans is no greater than that of agents included in Risk Group 3.

- An emergency plan which includes responses to biological, toxic or hazardous spills, fire, or life-threatening situations must be prepared, and reviewed by all personnel.
- A written reporting system for laboratory accidents and exposures must be in effect. A serum storage program for all laboratory and support personnel and a full medical surveillance and treatment program must be implemented.
- The Level 4 facility and its systems must be tested for containment capability upon completion of construction and annually thereafter.

4.5 ANIMAL BIOHAZARD CONTAINMENT FACILITIES

Laboratory facilities must provide containment for laboratory animals exposed to or harboring infectious agents which is appropriate to the risk level of the infectious agents involved. In addition to the physical plant requirements identified in Sections 4.1 to 4.4, special equipment (e.g. filter cages, partial or isolation caging systems) appropriate to the animal species as well as to the level of risk must be used.

Operational procedures for the care and maintenance of the infected animals must satisfy the Guidelines of the Canadian Council on Animal Care as well as the Laboratory Biosafety Guidelines in order to ensure not only protection for laboratory personnel and the environment but to ensure that every care is taken to avoid causing the animals unnecessary pain or suffering.

CHAPTER

5

LABORATORY DESIGN

This chapter is designed to inform building technologists, physical plant departments, laboratory management and cost estimators of the construction features required to achieve the four levels of containment outlined in Chapter 4.

A matrix system is used to present detailed information on the variety of design elements necessary for required biosafety standards, and to allow rapid comparison of features required to upgrade existing facilities.

5.1 LABORATORY LOCATION

The location of laboratories relative to other facilities in a building is one factor in containment. Proper location can help control entry by uninformed people. Lower and higher containment areas can be integrated to concentrate the necessary support services close to sterilizing and waste disposal facilities including exhaust air.

MATRIX N° 1

LABORATORY LOCATION

Containment Levels 1	2	3	4	Laboratory Location
●	●	●	●	Separated from public areas by door.
	●	●	●	Laboratory doors labelled with biohazard signs.
		●	●	Access limited to authorized personnel.
		●	●	In a separate sealed room with restricted access away from public thoroughfares.

●– MANDATORY
○– RECOMMENDED

MATRIX N° 1
(cont)

LABORATORY LOCATION

Containment Levels 1	2	3	4	Laboratory Location
		○	●	In a separate building or sealed room with independent air and exhaust – restricted access.
●	●			Office areas can be located within lab next to access or egress door.
		●	●	Office areas must be outside biocontainment facility.
		●	●	Facility must be kept locked when not in use (consistent with local fire and safety regulations).

●– MANDATORY
○– RECOMMENDED

5.2 PHYSICAL CONSTRUCTION OF CONTAINMENT BARRIERS

In addition to providing physical separation, the construction of the laboratory perimeter can also contribute to containment by providing surfaces that are easy to clean and disinfect. Careful consideration of long-term uses at the time of design and construction can allow for cost-effective upgrading, at minimal cost.

For high-level containment laboratories, the requirement for negative air pressure must be considered in the construction of walls and ceilings.

Users, designers and builders of these laboratories should be aware of the high costs involved in the construction and long-term maintenance of these facilities.

MATRIX N° 2

LABORATORY CONTAINMENT PERIMETER

Containment Levels 1	2	3	4	Laboratory Containment Perimeter
				A. WALLS
		○	●	Reinforced structural masonry.
		○	●	Reinforced non-load-bearing masonry.
		○	●	Steel frame reinforced non-load-bearing masonry.
		○	●	Reinforced concrete.
				B. CEILINGS
●	●			Steel frame gypsum partition or ceiling acoustic tile.
		●	●	Reinforced steel frame and gypsum ceiling, filler primer and paint finish.
				C. COATINGS AND SEALANTS
	○	●	●	Seamless, gas- and chemical-resistant wall and ceiling coatings.
	○	●	●	Chemical- and gas-resistant (disinfectant), non-hardening sealants.
	○	●	●	Containment seals for mechanical/electrical service openings.
				D. DOORS
	○	●	●	Doors to public areas lockable.
	○	●	●	Doors self-closing.

●– MANDATORY
○– RECOMMENDED

MATRIX N° 2
(cont)

LABORATORY CONTAINMENT PERIMETER

Containment Levels 1	2	3	4	D. Doors *(cont)*
			●	Pneumatic or compressible sealed doors electrically interlocked, airlock required for the separation of higher and lower biocontainment areas.
		○	●	Doors and frames of solid construction.
○	○	○	●	Door openings should be of sizes to allow passage of all anticipated equipment.
●	●	●	●	Doors to have required fire ratings and be located as for fire safety standards.
●	●	●	●	All exits marked and illuminated.
●	●	●	●	Egress to fire exits set out so that it is not necessary to travel through any high-hazard areas on exit route or to conform to applicable codes.
				E. WINDOWS
●	●			Windows if openable protected by fly screens.
		●	●	Windows of safety glass, solid stops sealed in place.
				F. FLOORS
●	●	●	●	Slip-resistant flooring.
○	○	●	●	Seamless, gas- and chemical-resistant epoxy coating with cove base.
●	●			Seamless, rolled or resilient tile flooring.

●– MANDATORY
○– RECOMMENDED

5.3 AIR-HANDLING SYSTEMS

Because of their insidious, pervasive nature, aerosols are a major factor in the dissemination of hazardous agents. They pose a risk to both the laboratory worker and the outside environment. Contamination by aerosols can be minimized by proper laboratory techniques, biological containment cabinets, setting and balancing the room air supply and exhaust systems and the use of HEPA filters.

It is of particular importance that purchased containment equipment (e.g. biological cabinets) be tested to meet specified standards after installation and that the connections between such equipment and room air handling systems should meet the standards required.

The following matrices indicate the HEPA filtration requirements for each containment level for supply and exhaust air from the laboratory and from biocontainment cabinet interior environments.

MATRIX N° 3 AIR HANDLING

Containment Levels 1	2	3	4	Air Handling
				A. ROOM AIR SUPPLY
		○	●	Air supply independent from adjoining facilities.
			●	Air supply HEPA-filtered.
	○	●	●	Directional inward, non-recirculated airflow.
		●	●	Interlocked with exhaust ventilation to prevent pressurization.
		○	●	Equipped with audible alarms to detect depressurization.
		○	●	Air supply ductwork sealed airtight and independent from others in facilities and accessible from outside containment laboratory.

●- MANDATORY
○- RECOMMENDED

MATRIX N° 3
(cont)

AIR HANDLING

Containment Levels				
1	2	3	4	**A. Room Air Supply *(cont)***
		●	●	Equipped with manual damper to permit sealing for decontamination procedures.
			●	Air supply system equipped with HEPA filters with magnehelic gauges.
				B. EXHAUST VENTILATION
		○	●	Sealed airtight independent-exhaust ductwork accessible from outside laboratory.
		○	●	All exhaust ventilation HEPA-filtered and connected to audible alarm.
	○	●	●	Interlocked with air supply to prevent pressurization.
	○	●	●	Equipped with manual damper to permit sealing for decontamination.
	○	●	●	Exhaust from laboratory at a minimum of ten room volumes per hour.
○	○	○	○	Air vertically discharged to the outside, clear of buildings or supply air intakes, at 12 metres/second.
●	●			Recirculated HEPA-filtered air permitted.
		●	●	Minimization of dead spaces where contaminated air can accumulate.
●	●	●	●	Ventilation sufficient to remove vapours of flammable liquids and dangerous chemicals before they reach hazardous concentrations.

●– MANDATORY
○– RECOMMENDED

MATRIX N° 3 *(cont)*

AIR HANDLING

Containment Levels 1	2	3	4	B. Exhaust Ventilation *(cont)*
		●	●	Exposed ductwork to stand clear of walls to allow access for maintenance, leak testing and access to equipment filters and lighting.
				C. BIOLOGICAL SAFETY CABINETS
	○	●		Class I
	○	●	●	Class II
			●	Class III
			●	Class I and II cabinets permitted with use of positive-pressure suits.
	●	●		Cabinet air can be recirculated in laboratory if HEPA-filtered.

●– MANDATORY
○– RECOMMENDED

5.3.1 REQUIREMENTS FOR BIOLOGICAL SAFETY CABINETS IN ALL LEVELS OF LABORATORIES

1. Room supply air system equipped with dampers to prevent backflow if biological safety cabinets are connected to exhaust ductwork.
2. If biological safety cabinets are connected to exhaust ductwork, connections are by thimble units where appropriate and room exhaust ducts are equipped with manual dampers to permit sealing for decontamination (ref. no. 26).
3. Cabinets to be located away from doors, from windows that can be opened, from room supply air and from heavily-travelled laboratory areas.
4. A minimum of 30cm clearance is recommended, where possible, behind each side of the cabinet to permit cleaning and testing and a minimum unobstructed clearance of 40cm at the exhaust filter discharge to permit testing.

5.3.2 REQUIREMENTS FOR HEPA FILTERS LEVELS 2-4 BIOCONTAINMENT LABORATORIES

1. All HEPA filters to have minimum particulate removal of 99.97% for particles of 0.3 μm.
2. HEPA filters installed as close as possible to the source of hazard to minimize length of contaminated ductwork.
3. HEPA filters to be installed in housings with leakproof junctions between filter frame and ducting.
4. HEPA filters to be installed in housings which will allow decontamination and Dioctylphthalate (DOP) testing in situ (i.e. access to both upstream and downstream face of filter) by the scanning method.
5. HEPA filters in biological safety cabinets to be monitored by magnehelic gauges.

5.4 DECONTAMINATION, STERILIZATION AND WASTE-DISPOSAL SYSTEMS

Biocontainment barriers should be maintained to the level required in all supply, drainage and waste-disposal systems, decontamination systems, sampling ports, and condensation collection and disposal systems as part of sterilization equipment.

In Canada, there are numerous pieces of legislation which control the disposal of infectious or contaminated biomedical waste. The waste generator is held directly accountable for ensuring that all stages of transportation and disposal are carried out in a safe and legal manner. Following decontamination, solid wastes are generally disposed of through burial in a landfill site. This process is usually regulated and controlled by the province. Liquid wastes which are released to the local sanitary sewer are controlled and regulated by municipal by-laws. When the effluent is released into the environment from a drain, septic tank, the local sewer or a sewage treatment plant, it comes under the jurisdiction of the province. The federal government has legislation which covers all areas not controlled by existing municipal or provincial legislation. In order to find out which rules apply to your operation, check with your municipal, provincial and federal authorities.

Most legislation does not refer specifically to communicable disease organisms by name. However, there

is usually some general requirement that wastes should not be or become a hazard to persons, animals or property. It is left to the waste generator to evaluate each risk and to ensure that it is not significant. The Animal Diseases and Protection Act and Regulations, 1987, name certain disease agents and also provide instructions for their disposal. These agents are those which are either not present in Canada or which are the subject of Agriculture Canada programs aimed at disease control or eradication. Communicable disease agents used for the production or field testing of veterinary biologics are also strictly controlled. For information, contact the Chief, Veterinary Biologics and Biotechnology, Health of Animals Directorate, Agriculture Canada, 3851 Fallowfield Road, Box 11300, Station H, Nepean, Ontario K2H 8P9 (tel: (613) 998-9320).

MATRIX N° 4

DECONTAMINATION, STERILIZATION AND WASTE DISPOSAL SYSTEMS

Containment Levels				
1	2	3	4	**Decontamination**
○	○	●	●	Laboratory floors, walls and ceilings to be treated with disinfectant-resistant, cleanable coatings.
○	○	●	●	Laboratory with all furnishings and surface materials disinfectant-resistant and cleanable.
		●	●	Laboratory perimeter sealed to allow gaseous decontamination of whole room.
				Sterilization
		○	●	Double-door pass-through autoclave in laboratory.
		○	●	Autoclave in laboratory.
○	○	●	●	Autoclave in facility.
●	●	●	●	Exposed steam pipes covered with insulating material.
			○	Incinerator in building.

●– MANDATORY
○– RECOMMENDED

MATRIX N° 4
(cont)

DECONTAMINATION, STERILIZATION AND WASTE DISPOSAL SYSTEMS

Containment Levels 1	2	3	4	Waste Disposal
				LIQUIDS
	○	●	●	Drainage traps filled with disinfectant specified by lab operator.
			●	All liquid effluents must be sterilized in mechanically and biologically monitored tanks located adjacent to the high-containment area prior to disposal to municipal sewers.
				SOLIDS
●	●	●	●	Provide space for stands supporting bags for collection of biomedical waste.
●				Provide space for a lockable, closed storage facility for biomedical waste which has to leave the laboratory for autoclaving or incineration.
			●	Provide space for disinfectant dunk tanks for all non-autoclavable materials and equipment exiting the high-containment area either as a part of the cabinet line or in the case of a suited lab, as part of the perimeter containment barrier.

●– MANDATORY
○– RECOMMENDED

5.5 PERSONAL HYGIENE AND SAFETY FACILITIES

Good laboratory practice and protective clothing minimize the contamination of laboratory personnel with infectious agents. The laboratory must, however, incorporate

facilities for changing into protective clothing, and for its eventual disposal, and also provide emergency personal decontamination and washing facilities required in the event of a spill or accident.

MATRIX N° 5

PERSONAL HYGIENE AND SAFETY FACILITIES

Containment Levels				
1	2	3	4	**Personal Hygiene and Safety Facilities**
●	●	●	●	Handwashing facilities in laboratory.
	○	●	●	Dedicated handwashing facilities with foot-operated taps in laboratory.
			●	Chemical deluge shower in laboratory area (for positive-pressure-suit mode).
○	○	○	●	Eye/face wash facilities equipped with in-use audio/visual alarm.
		○	●	Body shower in containment area.
		●	●	Clothing change area adjacent to containment area (0.5m^2 per person).
●	●	●	●	Provide storage space for laboratory clothing in lab or adjacent change area (minimum 300 linear mm. for each peg).
○	○	●	●	Provide space for laundry hamper adjacent to exit door (minimum 0.900m^2 for used laboratory clothing to be autoclaved prior to laundering.

●– MANDATORY
○– RECOMMENDED

5.6 BUILDING SERVICES

Plumbing and drainage, electrical, fire alarm and communications systems, other than those concerned with air handling, are covered in this section.

MATRIX N° 6 BUILDING SERVICES

Containment Levels 1	2	3	4	Building Services
				A. PLUMBING AND DRAINAGE
			●	All drains connected to sterilization system.
		○		All drains connected directly to sanitary sewer.
		●	●	All piping sealed with non-shrinking sealant at laboratory perimeter.
		●	●	Supply water services fitted with backflow preventers.
		●	●	Piping to be exposed and stand clear of walls within high containment area to allow access for maintenance.
		○	●	Main water supply control to be located outside laboratory.
●	●	●	●	All exposed hot and cold water pipes are to be covered with insulating material and protected from movement.
				B. COMPRESSED AIR AND GAS
	○	●	●	Air supply lines HEPA filtered or equivalent as backflow protection.
		●	●	All gas lines to have backflow preventers.

●– MANDATORY
○– RECOMMENDED

MATRIX N° 6
(cont)

BUILDING SERVICES

Containment Levels 1	2	3	4	B. Compressed Air and Gas *(cont)*
	○	●	●	All vacuum lines HEPA-filtered or equivalent.
		●	●	All supply line penetrations sealed with non-shrinking sealant at laboratory perimeter.
		○	●	No vacuum lines should lead from facility (needs met by pumps inside laboratory).
		○	●	Compressed gas cylinder storage outside laboratory.
				C. ELECTRICAL
		○	●	All supply conduit and control wiring to be sealed at the containment barrier with non-shrinking sealant.
		○	●	Fluorescent light ballasts and starters to be located outside biocontainment area.
		○	●	Circuit breakers located outside biocontainment area.
○	○	○	●	Building security systems integrated with laboratory safety and monitoring systems.
○	○	○	○	70 ft.-candles of light at work surface level (metric) minimum maintained at work surface.
●	●	●	●	All circuit-breaker switches and controls to be labelled.
	○	○	●	Electrical system is to be equipped with standby generator for emergency support of essential equipment.

●– MANDATORY
○– RECOMMENDED

MATRIX N° 6 *(cont)*

BUILDING SERVICES

Containment Levels 1	2	3	4	C. Electrical *(cont)*
●	●	●	●	Laboratory to be equipped with fire alarm system.
		○	●	Lab to be equipped with two-way intercom system between containment area and outside support area.
			○	Closed-circuit TV monitoring of entire work area of containment area.

5.7 EMERGENCY AND MONITORING PROVISIONS

Regular monitoring of containment barriers is essential to safe laboratory practice and to contingency plans to ensure that agents do not escape into the environment. Monitoring equipment, alarms, backup support systems, and access for the integration of testing equipment must be incorporated into the design.

MATRIX N° 7

EMERGENCY AND MONITORING PROVISIONS

Containment Levels 1	2	3	4	Emergency and Monitoring Provisions
				A. AIR HANDLING
	○	●	●	Directional inward non-recirculated airflow.
	○	○	○	Biological safety cabinets equipped with magnehelic gauges to monitor all HEPA filters.

●– MANDATORY
○– RECOMMENDED

MATRIX N° 7
(cont)

EMERGENCY AND MONITORING PROVISIONS

Containment Levels 1	2	3	4	A. Air Handling *(cont)*
●	●	●	●	Provision of access for decontamination of HEPA filters.
		○		Entry to laboratory via ventilated air-lock.
			●	Entry to laboratory via sealed air-lock.
		●	●	Rooms in isolation area to be maintained at pressure negative to corridor with greatest negative pressure in most hazardous room.
				B. FIRE PREVENTION AND CONTAINMENT
●	●	●	●	Equipped with fire alarms.
●	●	●	●	Equipped with fire extinguishers.
●	●	●	●	Doors to have appropriate fire ratings.
●	●	●	●	All fire exits marked and illuminated.
			○	Biocontainment area designated as "burn out". Firemen to enter only to save life, not to extinguish fire, which should be controlled from without, to prevent spread.
●	●	●	●	Equipped with suitable storage cabinets or explosion-proof refrigerators which are clearly labelled for flammable liquids.
○	○	○	○	Storage for flammable liquids to be located outside biocontainment area.

●– MANDATORY
○– RECOMMENDED

MATRIX N° 7
(cont)

EMERGENCY AND MONITORING PROVISIONS

Containment Levels 1	2	3	4	Emergency and Monitoring Provisions
				C. PERSONNEL EMERGENCY EQUIPMENT
		○	●	Equipped with positive-pressure hood respirators with compressed air cylinders in support area.
●	●	●	●	Eye/face wash facilities in laboratory.
		○	●	Deluge or body shower in support area.
		○	●	Equipped with voice intercom system between high-containment zone and support area.
●	●	●	●	Emergency lighting.
				D. BACKUP SERVICE
		○	●	Equipped with standby generator for support of essential equipment.

●– MANDATORY
○– RECOMMENDED

5.8 COMMISSIONING

An institution in which work requiring containment facilities is carried out must bear a major part of the responsibility for ensuring that the facilities, and the work in them, meet acceptable standards. Therefore, before putting a containment laboratory into service, it is essential that the institution satisfy itself that the laboratory meets the standards required. The laboratory equipment and procedures must pass a series of inspections, testing and proving standards specified in their design and construction before agents are used in the laboratory. This inspection must be repeated at least yearly thereafter. Detailed records of inspection and test results should be maintained by the institution indefinitely.

MATRIX N° 8

COMMISSIONING

Containment Levels 1	2	3	4	Commissioning
				Construction of laboratory perimeter to be leakproof and able to withstand loading characteristics imposed by negative air pressure required in laboratory operation:
		●		a) Integrity demonstrated by visual inspection.
			●	b) Integrity demonstrated by physical testing (pressure decay 0.05 wg loss/min) at 2"wg.
		○	●	All air supply and exhaust ductwork tested to be leak-tight by pressure decay*: level 3 not >0.2% duct vol./min. at 2"wg (500Pa); level 4 not > 0.1% duct vol./min. at 2"wg (500Pa)
		●	●	All air supply and exhaust ductwork verified to have back-draft protection.
		●	●	All HEPA filters tested to meet required specification after installation.
		○	●	All HEPA-filter housings tested to be leak tight*: not >0.2% of vol./min. at 10"wg. (2500Pa).
	●	●	●	Testing of biological safety cabinets meets required specifications after installation.
●	●	●	●	Testing of autoclaves to meet specified standards after installation by the use of biological indicators.

*ANSI/ASME N510 (see bibliography no. 4)

●- MANDATORY
○- RECOMMENDED

MATRIX N° 8
(cont)

COMMISSIONING

Containment Levels				
1	2	3	4	**Commissioning**
			●	Drainage and liquid-waste-disposal systems including sampling ports tested to ensure efficacy by use of biological indicators.
		●	●	Verification of alarm systems for air systems failure (exhaust, supply, room pressure).
	●	●	●	Verification of alarm systems for electrical failure.
●	●	●	●	Verification of fire alarm systems.
		○	●	Verification of voice or visual intercom systems.
	○	●	●	Testing of directional airflow demonstrated by field tests with visual smoke.

●– MANDATORY
○– RECOMMENDED

APPENDIX

A

SAFETY EQUIPMENT AND BIOLOGICAL SAFETY CABINETS

SAFETY EQUIPMENT

An essential element in maintaining personal safety and environmental protection is the correct selection, use and maintenance of safety equipment in the laboratory. Items of safety equipment must be well maintained and regularly serviced. There must also be a regular program of testing and inspection, and accurate records must be kept.

Biological safety cabinets must be certified by trained technicians when newly installed and annually thereafter. They must be decontaminated before removal.

The following is a list of safety devices appropriate to the containment laboratory:

Type	Application
Animal cages or boxes	partial to total containment of aerosols – provides protection from cross-contamination and environmental and personnel protection
Autoclaves	heat sterilization
Biological Safety Cabinets	
Class I	aerosol control personnel and environmental protection
Class II	personnel, product and environmental protection
Class III	personnel and environmental protection
Blenders – mixers	aerosol-free blenders provide containment of aerosols
Centrifuge equipment	safety trunnion cups provide containment of aerosols

Type	Application
Face and eye protection	safety glasses, goggles and full-face shields provide protection from flying objects, chemicals and biological splashes
Gloves	provide hand protection of varying degrees – check technical specifications to determine degree of protection
HEPA filters	available in various sizes, including cartridge-disposable, provide 99.97% removal of 0.3μm particulates
Incinerators – micro	electric or gas with side-arm to contain splatters from loops
Laboratory clothing	coats, gowns or ventilated suits appropriate to hazard
Leakproof containers	variety of containers preferably of stainless steel which are autoclavable and have tight-fitting lid which may be used for transporting infectious materials to autoclave
Pipetting devices	variety of devices are available which eliminate need to pipette by mouth
Respiratory protection	partial or full-face protection is provided with variety of filters including HEPA
Sharps	puncture-proof containers which are autoclavable are used to discard used hypodermic syringes and needles
Sterility indicators	indicator devices with heat-resistant spores are used for determining autoclave efficacy

APPENDIX

B

BIOLOGICAL SAFETY CABINETS

Biological safety cabinets are the most widely used and accepted primary containment devices. Class I or II cabinets can provide partial containment, and Class III cabinets have the potential for complete containment. Biological safety cabinets must not be confused with other laminar flow devices or 'clean benches'; in particular, horizontal flow cabinets which direct air towards the operator should not be used for handling infectious, toxic or sensitizing materials.

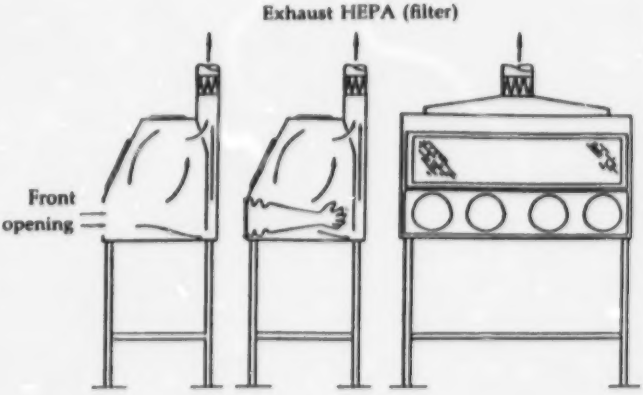
The correct location, installation, and certification of the biological safety cabinet is critical to its performance in containing aerosols. Cabinets must be annually inspected and tested by trained service personnel to CSA Z316.3-M87 or its subsequent modification.

Laboratory personnel must be trained in the correct use and maintenance of biological safety cabinets to ensure that personnel and product protection (where applicable) are maintained.

Class I Biological Safety Cabinet

This is a ventilated cabinet for personnel protection with an unrecirculated inward airflow away from the operator. This unit should be fitted with a HEPA filter to protect the environment from discharged agents.

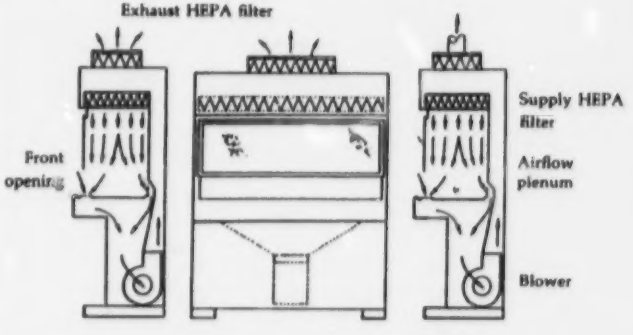
Schematic Diagram of a Class I Biological Safety Cabinet



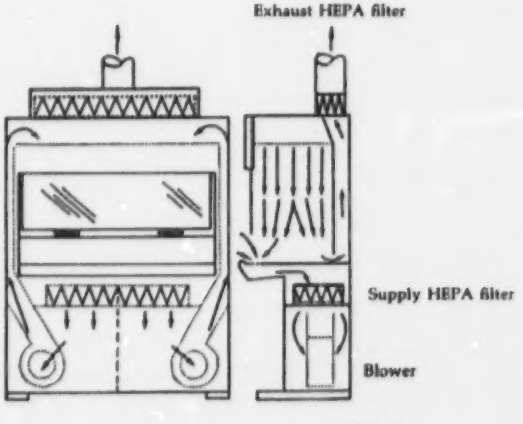
Class II Biological Safety Cabinet

This is a ventilated cabinet for personnel, product and environmental protection which provides inward airflow and HEPA-filtered supply and exhaust air. The Class II cabinet has four designs: one recirculates most of the air; a second exhausts most of the air; a third provides total exhaust; and a fourth includes cabinets which are convertible between the other designs. Class II cabinets may be of use with low to moderate risk biological agents, minute quantities of toxic chemicals, and trace quantities of radionuclides; however, care must be exercised in selecting the correct Class II cabinet design for these purposes.

Schematic Diagram of a Class II Biological Safety Cabinet



Type A

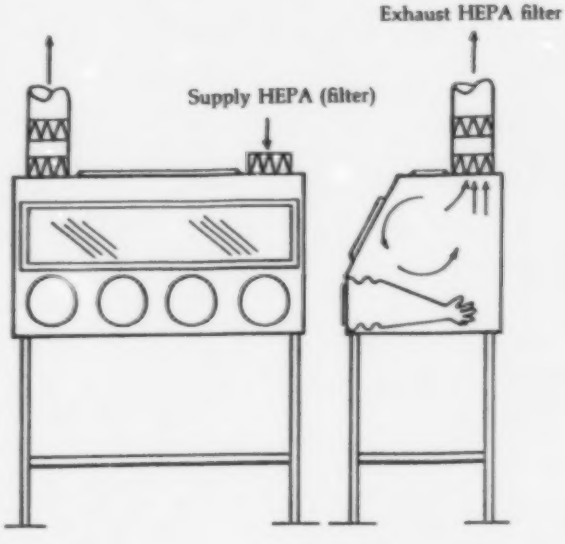


Type B

Class III Biological Safety Cabinet

A Class III cabinet is a totally enclosed ventilated cabinet which is gas-tight, and maintained under negative air pressure (0.5 inches water gauge). The supply air is HEPA-filtered and the exhaust air has two HEPA filters in series. Work is performed in the cabinet by the use of attached rubber gloves.

Schematic Diagram of a Class III Biological Safety Cabinet



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